

#### **WHAT IS CLAIMED IS:**

1. A stromatolysing reagent for use in the determination of at least two leukocyte populations in blood, two such populations being lymphoid and myeloid, the blood being processed through a blood cell analyzer using the Coulter Principle of operation, which employs a leukocyte sensing zone, the blood having been first diluted with an electrically conductive isotonically balanced diluent, wherein said stromatolysing reagent comprises an aqueous solution of a tetraalkylammonium halide salt where the alkyl group is selected from the class consisting of dodecyl, tetradecyl and hexadecyl and a trialkylammonium halide salt where the alkyl group is selected from the class consisting of alkyl radicals having 8-12 carbons having surface active properties, said salts being present in sufficient amounts for positioning said leukocyte populations relative to one another and relative to a blood cell volume reference point, within the time constraints of said blood cell analyzer.
2. A stromatolysing reagent according to claim 1 wherein there is included in the aqueous solution a further quarternary ammonium salt having the characteristics of a 2-hydroxycetyl-2-hydroxyethyldimethylammonium halide salt.
3. A stromatolysing reagent according to claim 1 wherein there is included in the aqueous solution a 2-hydroxycetyl-2-hydroxyethyldimethylammonium halide salt.
4. A method of determining leukocytes and hemoglobin in blood comprising using a lysing agent containing an aqueous solution of a tetraalkylammonium halide salt where the alkyl group is selected from the class consisting of dodecyl, tetradecyl and hexadecyl and a trialkylammonium halide salt where the alkyl group is selected from the class consisting of alkyl radicals having 8-12 carbons having surface active properties to stromatolyze erythrocytes and platelet cells and to convert hemoglobin to a chromagen.

5. A method according to claim 4 wherein the aqueous solution further includes a quarternary ammonium salt having the characteristics of a 2-hydroxycetyl-2-hydroxyethyldimethylammonium halide salt.
6. A method according to claim 4 wherein the aqueous solution further includes a 2-hydroxycetyl-2-hydroxyethyldimethylammonium halide salt.
7. A kit of a lytic reagent system for selective chemical treatment of a whole blood sample comprising:
  - (a) a lytic reagent composition comprising saponin and a lytic reagent, wherein said lytic reagent is composed of an acid compound selected from the class consisting of 2-chloro- and 2-fluoroacetic acids, 2-hydroxyacetic acid (glycolic acid), 2,3-dihydroxypropanoic acid (glyceric acid), 1,5-pentandioic acid (glutaric acid), 2,3,4,5,6-pentahydroxyhexanoic acid (gluconic acid), sodium pyrosulfate, sodium hydrogen sulfate (bisulfate), potassium pyrosulfate, potassium hydrogen sulfate (bisulfate), 1-butanefulfonic acid sodium salt, and mixtures thereof; wherein the relative concentration of the lytic reagent composition is in an effective amount to effect partitioning of a whole blood sample into a lysed red cell fraction and a leukocyte fraction by (i) causing rapid and essentially complete hemolysis of red blood cells in the blood sample and, (ii) inducing changes in leukocytes in said blood sample to enhance the ability of instrumentation to perform differential analysis and identification of at least five sub-populations of leukocytes, and
  - (b) a quench reagent, said quench reagent being present in sufficient concentration to retard the lytic activity of the lytic reagent composition and restore the physiological environment of the leukocytes within the sample.
8. A kit of a lytic reagent system for selective chemical treatment of a whole blood sample comprising:
  - (a) a differentiation effective amount of a lytic reagent and saponin, wherein said lytic reagent is composed of an acid compound selected from the class

consisting of 2-chloro- and 2-fluoroacetic acids, 2-hydroxyacetic acid (glycolic acid), 2,3-dihydroxypropanoic acid (glyceric acid), 1,5-pentandioic acid (glutaric acid), 2,3,4,5,6-pentahydroxyhexanoic acid (gluconic acid), sodium pyrosulfate, sodium hydrogen sulfate (bisulfate), potassium pyrosulfate, potassium hydrogen sulfate (bisulfate), 1-butanefulfonic acid sodium salt, and mixtures thereof; and wherein the differentiation effective amount of said lytic reagent, when added to a whole blood sample, effecting (i) a decrease in the pH of the sample from its physiological level to a pH in the range of from about 1.8 to about 6.0 while maintaining the osmolality of the fluid at less than about 150 mOs, (ii) rapid and essentially complete hemolysis of the red blood cell fraction in the blood sample, and (iii) changes in the leukocyte cell fraction of the blood sample to enhance the ability of instrumentation to perform differential analysis and identification of at least five sub-populations of leukocytes; and

(b) a quench reagent, said quench reagent being present in sufficient concentration to retard the lytic activity of the lytic reagent composition and restore the physiological environment of the leukocytes within the sample.

9. A kit of a lytic reagent system for white blood cell differential analysis comprising:

(a) a lytic reagent composition for selective chemical treatment of a whole blood sample, said composition comprising saponin and a lytic reagent; wherein said lytic is a reagent acid compound selected from the class consisting of 2-chloro- and 2-fluoroacetic acids, 2-hydroxyacetic acid (glycolic acid), 2,3-dihydroxypropanoic acid (glyceric acid), 1,5-pentandioic acid (glutaric acid), 2,3,4,5,6-pentahydroxyhexanoic acid (gluconic acid), sodium pyrosulfate, sodium hydrogen sulfate (bisulfate), potassium pyrosulfate, potassium hydrogen sulfate (bisulfate), 1-butanefulfonic acid sodium salt, and mixtures thereof; wherein the relative concentration of the lytic reagent composition is in an effective amount to effect partitioning of a whole blood sample into a lysed red cell fraction and an essentially intact leukocyte fraction in such a state as to allow differential analysis of at least five populations of such leukocytes; and

(b) a quench reagent, said quench reagent being present in sufficient concentration to retard the lytic activity of the lytic reagent composition and restore the physiological environment of the leukocytes within the sample.

10. A lytic reagent composition for selective chemical treatment of a whole blood sample, said lytic reagent composition comprising saponin and a lytic reagent wherein said lytic reagent is an acid compound selected from the class consisting of 2-chloro- and 2-fluoroacetic acids, 2-hydroxyacetic acid (glycolic acid), 2,3-dihydroxypropanoic acid (glyceric acid), 1,5-pentandioic acid (glutaric acid), 2,3,4,5,6-pentahydroxyhexanoic acid (gluconic acid), sodium pyrosulfate, sodium hydrogen sulfate (bisulfate), potassium pyrosulfate, potassium hydrogen sulfate (bisulfate), 1-butanesulfonic acid sodium salt, and mixtures thereof; and wherein the relative concentration of the lytic reagent composition is in an effective amount to effect partitioning of a whole blood sample into a lysed red cell fraction and an essentially intact leukocyte fraction in such a state as to allow differential analysis of at least five sub-populations of such leukocytes.

11. A lytic reagent composition for selective chemical treatment of a whole blood sample, said lytic reagent composition comprising saponin and a lytic reagent, wherein said lytic reagent is an acid compound selected from the class consisting of 2-chloro- and 2-fluoroacetic acids, 2-hydroxyacetic acid (glycolic acid), 2,3-dihydroxypropanoic acid (glyceric acid), 1,5-pentandioic acid (glutaric acid), 2,3,4,5,6-pentahydroxyhexanoic acid (gluconic acid), sodium pyrosulfate, sodium hydrogen sulfate (bisulfate), potassium pyrosulfate, potassium hydrogen sulfate (bisulfate), 1-butanesulfonic acid sodium salt, and mixtures thereof; and wherein the relative concentration of the lytic reagent composition is in an effective amount to effect partitioning of a whole blood sample into a lysed red cell fraction and a leukocyte fraction by (i) causing rapid and essentially complete hemolysis of red blood cells in the blood sample and. (ii) inducing changes in leukocytes in said blood sample to enhance the ability of instrumentation to

perform differential analysis and identification of at least five (5) sub-populations of leukocytes.

12. A lytic reagent composition for selective chemical treatment of a whole blood sample. said lytic reagent composition comprising a differentiation effective amount of a lytic reagent and saponin, wherein said lytic reagent is an acid compound selected from the class consisting of 2-chloro- and 2-fluoroacetic acids, 2-hydroxyacetic acid (glycolic acid), 2,3-dihydroxypropanoic acid (glyceric acid), 1,5-pentandioic acid (glutaric acid), 2,3,4,5,6-pentahydroxyhexanoic acid (gluconic acid), sodium pyrosulfate, sodium hydrogen sulfate (bisulfate), potassium pyrosulfate, potassium hydrogen sulfate (bisulfate), 1-butanesulfonic acid sodium salt, and mixtures thereof; and wherein the differentiation effective amount of said lytic reagent composition, when added to a whole blood sample, effecting (i) a decrease in the pH of the sample from its physiological level to a pH in the range of from about 1.8 to about 6.0 while maintaining the osmolality of the fluid at less than about 150 mOs, (ii) rapid and essentially complete hemolysis of the red blood cell fraction in the blood sample, and (iii) changes in the leukocyte cell fraction of the blood sample to enhance the ability of instrumentation to perform differential analysis and identification of at least five (5) subpopulations of leukocytes.

13. A process for identifying classes, or classes and also selected subclasses of leukocytes, which process includes the steps of: treating a sample containing whole blood with a reagent in which an acid compound selected from the class consisting of 2-chloro- and 2-fluoroacetic acids, 2-hydroxyacetic acid (glycolic acid), 2,3-dihydroxypropanoic acid (glyceric acid), 1,5-pentandioic acid (glutaric acid), 2,3,4,5,6-pentahydroxyhexanoic acid (gluconic acid), sodium pyrosulfate, sodium hydrogen sulfate (bisulfate), potassium pyrosulfate, potassium hydrogen sulfate (bisulfate), 1-butanesulfonic acid sodium salt, and mixtures thereof, is an active ingredient; and then identifying the treated leukocytes by at least optical detection.

14. A multi-purpose isotonic diluent reagent comprising a mixture of an aqueous solution of one or more alkali metal salts which dissociate into individual ions for establishing the majority of the isotonicity, osmolality and conductivity of the reagent, and one or more additives comprising minor amounts of a buffering agent, a chelating agent, an anesthetic agent, and a germicidal agent, the diluent having a pH of 6.9 to 7.1, and osmolality of 292 to 302 milliosmoles/kg and a conductivity 18 to 20 milliSiemens/cm.

15. A multi-purpose isotonic diluent reagent according to claim 14 wherein the alkali metal salt is selected from the class consisting of sodium chloride, potassium chloride, sodium sulfate, potassium sulfate, sodium nitrate and potassium nitrate.

16. A multi-purpose isotonic diluent reagent according to claim 14 wherein the alkali metal salt is comprised of a major alkali salt and a minor alkali metal salt with the ratio of the major alkali metal salt to the minor alkali metal salts being at least 8:1 to about 10:1.

17. A multi-purpose isotonic diluent reagent according to claim 16 wherein the major alkali metal salt is sodium sulfate and the minor alkali metal salt is sodium chloride.

18. A stromatolysing reagent for use in the determination of at least two leukocyte populations in blood, two such populations being lymphoid and myloid, the blood being processed through a blood cell analyzer, which employs a leukocyte sensing zone, the blood having been first diluted with an electrically conductive, optically clear and isotonically balanced diluent, wherein said stromatolysing reagent comprises an aqueous solution of a tetraalkylammonium halide salt, wherein the alkyl groups are selected from the class of alkyl and modified alkyl groups, consisting of methyl, dodecyl, tetradecyl, hexadecyl, 2-hydroxyhexadecyl and 2-hydroxyethyl, and mixtures thereof, and a trialkylammonium halide salt wherein the alkyl group is selected from the class consisting of alkyl radicals having 8-12 carbons and having surface

active properties, said salts being present in sufficient amounts for positioning said leukocyte populations relative to one another and relative to a blood cell volume reference point, within the time constraints of said blood cell analyzer.

19. A stromatolysing reagent according to claim 18 wherein there is included in the aqueous solution one or more compounds selected from the class methyl benzoate, ethyl benzoate, propyl benzoate, methyl paraben (methyl *p*-hydroxybenzoate), ethyl paraben, propyl paraben, methyl 2,4-dihydroxybenzoate, ethyl 2,4-dihydroxybenzoate, propyl 2,4-dihydroxybenzoate and similar compounds.

20. A stromatolysing reagent according to claim 18 wherein there is included in the aqueous solution methyl paraben.

21. A method of determining leukocytes and hemoglobin in blood comprising using a lysing agent containing an aqueous solution of a tetraalkylammonium halide salt where the alkyl group is selected from the class consisting of methyl, dodecyl, tetradecyl, hexadecyl, 2-hydroxyhexadecyl and 2-hydroxyethyl, and mixtures thereof, and a trialkylammonium halide salt wherein the alkyl group is selected from the class consisting of alkyl radicals having 8-12 carbons and having surface active properties to stromatolyse erythrocytes and platelet cells and to convert hemoglobin to a chromagen.

22. A method according to claim 21 wherein the aqueous solution further includes one or more compounds selected from the class methyl benzoate, ethyl benzoate, propyl benzoate, methyl paraben (methyl *p*-hydroxybenzoate), ethyl paraben, propyl paraben, methyl 2,4-dihydroxybenzoate, ethyl 2,4-dihydroxybenzoate, propyl 2,4-dihydroxybenzoate and similar compounds, for the purpose of increasing the specific absorbance of the formed hemoglobin chromogen.

23. A method according to claim 21 wherein the aqueous solution further includes methyl paraben.

24. A method which comprises the steps of:

- I. treating the blood sample with an isotonic diluent comprising an aqueous solution of one or more alkali metal salts which disassociate into individual ions for establishing the majority of the isotonicity, osmolality and conductivity of the reagent, and additives comprising one or more minor amounts of a buffering agent, a chelating agent, an anesthetic agent, and a germicidal agent, the diluent being at a pH of 6.9 to 7.1, an osmolality of 292 to 302 milliosmoles/kg and a conductivity 18 to 20 milliSiemens/cm, and
- II. lysing with a reagent comprising a mixture of an aqueous solution of a tetraalkylammonium halide salt where the alkyl group is selected from the class consisting of methyl, dodecyl, tetradecyl, hexadecyl, 2-hydroxyhexadecyl and 2-hydroxyethyl, and mixtures thereof, and a trialkylammonium halide salt wherein the alkyl group is selected from the class consisting of alkyl radicals having 8-12 carbons and having surface active properties, said salts being present in a concentration range which is effective to give a differential determination of lymphoid and myeloid populations of leukocytes followed by determination of hemoglobin values, particularly in automatic particle counting systems.

25. The method of claim 24 wherein the lysing reagent further includes one or more compounds selected from the class methyl benzoate, ethyl benzoate, propyl benzoate, methyl paraben (methyl *p*-hydroxybenzoate), ethyl paraben, propyl paraben, methyl 2,4-dihydroxybenzoate, ethyl 2,4-dihydroxybenzoate, propyl 2,4-dihydroxybenzoate and similar compounds for the purpose of increasing the specific absorbance of the formed hemoglobin chromogen.